[0012] The molecular linker links the polymerizing agent to one or more chemical moieties that are capable of reversibly binding to the template (or target) strand of a nucleic acid molecule. In particular examples, the molecular linker maintains the polymerizing agent and the chemical moieties sufficiently spaced a distance from one another to avoid substantial entanglement of the polymerizing agent and the chemical moieties in the absence of the target or template nucleic acid molecule, while allowing interaction of the polymerizing agent and the chemical moieties in the presence of the target nucleic acid molecule. For example, the molecular linkers can be spaced around the polymerizing agent a sufficient distance to inhibit entanglement of the linkers, and are of sufficient length to reach the active site of the polymerizing agent. In some examples, the molecular linker (or at least a portion thereof) is of sufficient rigidity to reduce interaction of the polymerizing enzyme and the chemical moieties in the absence of the target nucleic acid molecule.

[0013] The molecular linker (or a portion thereof, such as a molecular rod that is part of the molecular linker) has a sufficient length in view of its flexibility to space the polymerizing agent and the chemical moieties sufficiently apart to avoid the undesired interaction in the absence of the target nucleic acid molecule, but retain sufficient flexibility to allow the polymerizing agent and the chemical moieties to interact with each other and with the target nucleic acid molecule, for example when the polymerizing agent binds to the target nucleic acid molecule. For example, at least part of the molecular linker can have a persistence length that permits at least a portion of the molecular linker to be of sufficient rigidity and length to reduce interaction of the polymerizing agent (such as a tag associated with the polymerizing agent) and the chemical moieties in the absence of the target nucleic acid molecule, and allows interaction of the polymerizing agent and the chemical moieties in the presence of the target nucleic acid molecule.

[0014] In particular examples, the total length of the molecular linker is different than (such as greater or less than) the persistence length of one or more components that make up the linker, such as a double- or single-stranded nucleic acid molecule. However, in particular examples, the total length of the molecular linker does not exceed a length beyond which significant interaction occurs between the polymerizing agent and the chemical moieties in the absence of the target nucleic acid molecule, while allowing significant interaction of the polymerizing agent and the chemical moieties, as well as the target nucleic acid molecule, in the presence of the target nucleic acid molecule. Such interactions can be measured using methods known in the art, for example by measuring acceptor emission fluorescence when the polymerizing agent includes a donor fluorophore and one or more chemical moieties include a corresponding acceptor fluorophore of a FRET pair. In other examples, a polymerizing agent is substantially maintained at a distance of at least twice the Förster radius (such as a Förster radius of 22 to 90 Å) from the chemical moieties in the absence of the target.

[0015] Persistence length (lp) is the average local conformation for a linear chain, which reflects the sum of the average projections of all chain segments on a direction described by a given segment. Therefore, persistence length is a measure of the rigidity or stiffness of a polymer chain. In particular examples, persistence length is the degree of bending (and hence the effective stiffness of the chain) which, in effect, measures the contour distance over which there occurs, on the average, a 68.40° bend. Therefore, the persistence length will vary depending on the composition of the molecular linker. For example, the persistence length for a double-stranded DNA (dsDNA) molecule will differ from that of a single-stranded DNA (ssDNA) molecule and from polyethylene glycol (PEG). In particular examples, dsDNA has a persistence length of about 400-500 Å (such as 450-500 Å), and dsRNA has a persistence length of 700-750 Å, for example at an ionic strength of about 0.2 M and at a temperature of 20° C. In particular examples, ssDNA has a persistence length of about 40 Å (for example at 20° C.) (Clossey and Carlon, *Phys. Rev. E. Stat. Nonlin. Soft. Matter. Phys.* 68(6 Pt 1):061911, 2003). In particular examples, PEG has a persistence length of about 3.8 Å.

[0016] In particular examples, the molecular linkers include linear polymers, such as polymers of nucleic acids, amino acids, sugar, PEG, or combinations thereof. For example, molecular linkers include, but are not limited to, tethers, molecular rods, or combinations thereof. For example, the molecular linker of sufficient rigidity can include a molecular rod, for example a molecular rod composed of a dsDNA. In some examples, the molecular linker of sufficient rigidity includes multiple molecular rods linked by tethers, or multiple tethers linked by molecular rods. One particular example of a tether is a molecule composed of (or in some examples consisting of) polyethylene glycol (PEG).

[0017] The polymerizing agent and the chemical moieties can be linked in a spatially separated orientation by one or more molecular linkers so that the polymerizing agent and the chemical moieties do not interact to provide the reaction in the absence of the target nucleic acid molecule. However, the molecular linker permits the polymerizing agent and the chemical moieties, under predetermined conditions, to be brought into sufficient proximity with one another to interact and produce a predetermined reaction, such as a detectable signal or interaction with the target nucleic acid molecule. For example, at least one of the tags associated with the polymerizing agent or the chemical moiety can be activated when brought into sufficient proximity to another tag, such as the excitation of an acceptor fluorophore tag by a donor fluorophore tag when the donor and acceptor are in sufficient proximity with one another.

[0018] Also provided by the present disclosure is a polymerizing agent that includes an active site capable of binding to a target nucleic acid molecule and promoting synthesis of a complementary nucleic acid molecule that elongates as complementary nucleotides are incorporated into the complementary nucleic acid molecule. The polymerizing agent further includes one or more molecular linkers spaced apart on the polymerizing agent to inhibit entanglement, wherein each linker carries a different chemical moiety (such as a nonhydrolyzable nucleotide analog) that is capable of reversibly binding to the template strand of a nucleic acid molecule, without being detached from the linker, by specifically binding with a complementary nucleotide in the target nucleic acid molecule. In particular examples, the polymerizing agent further includes a tag associated with each chemical moiety that identifies the chemical moiety carried by the linker. In addition, the polymerase can be associated with a tag that interacts with the tag associated with the chemical moiety to emit a characteristic signal that identifies the chemical moiety carried by the linker.

[0019] Also provided by the present disclosure are methods of using the disclosed nanoprobes, for example to determine